THE AMERICAN JOURNAL OF GASTROENTEROLOGY

Published by Elsevier Science Inc.

Vol. 97, No. 8, 2002
ISSN 0002-9270/02/$22.00
PII S0002-9270(02)04271-5

(6) Crohn's disease

Anti–Tumor Necrosis Factor Treatment Restores the Gut Barrier in Crohn's Disease

Peter Suenaert, M.D., Veerle Bulteel, M.Sc., Liesbeth Lemmens, M.Sc., Maja Noman, M.D., Benny Geypens, M.Sc., Ph.D., Gert Van Assche, MD., Ph.D., Karel Geboes, M.D., Ph.D., Jan L. Ceuppens, M.D., Ph.D., and Paul Rutgeerts, M.D., Ph.D.

Department of Gastroenterology, Department of Pathology, Laboratory of Experimental Immunology, University Hospital Gasthuisberg, Catholic University Leuven, Leuven, Belgium

OBJECTIVES: A primary defect of the tight junctions and, hence, increased intestinal epithelial permeability has been proposed as a basic pathogenic event in Crohn's disease. Challenge of the mucosal immune system by the commensal gut flora would then result in chronic inflammation. Alternatively, increased permeability could be the result of inflammation. Our aim was to study intestinal permeability in refractory Crohn's disease before and after treatment with monoclonal chimeric antibodies directed against tumor necrosis factor (TNF) to investigate whether the abnormal permeability persists after control of inflammation.

METHODS: Twenty-three patients with active Crohn's disease were evaluated before and 4 wk after a single infusion of 5 mg/kg infliximab. Intestinal permeability was studied by measurement of urinary excretion of ^{51}Cr-EDTA after oral intake.

RESULTS: The increased permeation of ^{51}Cr-EDTA through the small intestine (1.63% interquartile range [IQR] 1.06–2.07) and the overall permeation (3.27% IQR 2.40–4.38) before therapy decreased significantly after infliximab infusion to values (1.04% IQR 0.74–1.54 and 2.42% IQR 2.03–2.80, respectively) in the range of those found in normal volunteers (1.12% IQR 0.85–1.58 and 2.28% IQR 1.88–2.86, respectively).

CONCLUSION: Inhibiting the proinflammatory cytokine tumor necrosis factor dramatically reduces gut inflammation and largely restores the gut barrier in Crohn's disease. Our data confirm the central role of TNF in gut barrier modulation in inflammatory conditions *in vivo*. (Am J Gastroenterol 2002;97:2000–2004. © 2002 by Am. Coll. of Gastroenterology)

INTRODUCTION

In 1972 Shorter *et al.* (1) raised the hypothesis that the basic defect in Crohn's disease (CD) might be an abnormal epithelial gut barrier consisting of a single layer of columnar epithelial cells joined together by tight junctions (2). Increased passage of antigens through the intestinal mucosa then would inadequately activate the immune system, leading to chronic gut inflammation. The concept of a "permeable" epithelial intestinal barrier in Crohn's disease has gained much support since. Moreover, ultrastructural studies using freeze fracture have shown abnormalities of the tight junctions even in apparently unaffected bowel segments of patients with Crohn's disease (3). Experimental studies in rats have shown that tight junctional permeation of ^{51}Cr-EDTA (^{51}Cr-labeled ethylenediaminetetra-acetate) correlates well with the uptake of macromolecules in inflamed segments of small bowel (4).

An important step in permeability research in Crohn's disease was the development of noninvasive methods for measuring permeability *in vivo*. Intestinal barrier dysfunction in inflammatory bowel disease (IBD), as evidenced by increased ^{51}Cr-EDTA permeation, was first demonstrated by Bjarnason *et al.* in 1983 (5). Teahon *et al.* further validated this method (6). These authors found a good relationship between the site of increased ^{51}Cr-EDTA permeation and the location of the disease in the intestine. An abnormal overall gut permeability has since been documented in a large proportion of the patients with CD in many studies (7–12). Increased permeation of macromolecules (13) as well as of ^{51}Cr-EDTA (14) has also been demonstrated in macroscopically noninvolved bowel in patients with Crohn's disease.

In 1986, Hollander *et al.* (15) found increased intestinal permeability for PEG-400 in a proportion of first degree relatives of Crohn's disease patients. This finding has been confirmed for all other probes, and it is now well established that 10–25% of first degree relatives of Crohn's disease patients also have a leaky gut (16–18). These findings point toward a primary defect that might be genetically inherited.

However, several studies showed that intestinal permeability is restored when remission is obtained in many patients with active Crohn's disease by treatment with an elemental diet (19–21).

Prednisolone therapy also may decrease permeability in active Crohn's disease and ulcerative colitis in children and adolescent patients (22). These data suggest a close relationship between inflammatory activity and gut permeability. Moreover, some studies suggest that a persisting in-

Table 1. Crohn's Disease Patient Data

Age at onset (yr)	25 (range 12–67)
Disease location	No. of patients
Ileitis	6
Ileocolitis	11
Colitis	6
Previous surgery	7
Therapy	
Glucocorticosteroids	13
Immunosuppressives (azathioprine)	5
Glucocorticosteroids & immunosuppressives	3
Mesalazine/salazopyrine	11/1

creased permeability is a risk factor for relapse in Crohn's disease (23).

The antigenic trigger most likely involved in activation of the gut immune system is the commensal gut flora. This flora is critical for the development of chronic inflammation in most models of IBD (24). Also in Crohn's disease in human patients, the luminal flora, their products, and dietary antigens play a key role in the initiation of early lesions, in the modulation of the activity of the disease, and in the perpetuation of the inflammation (25–28). The question thus remains whether patients with CD have a leaky gut to start with, or whether the increased permeability is the consequence of inflammation.

Our hypothesis was that reducing the inflammation with monoclonal antibodies directed against tumor necrosis factor (TNF) might restore the barrier dysfunction associated with inflammation or expose a pre-existing underlying gut barrier defect.

The present study shows that chimeric monoclonal antibodies directed against TNF restore the gut barrier, and thus confirms that TNF plays a key role in gut barrier function *in vivo* in inflammatory conditions. This study also shows that inflammation and gut permeability are closely interrelated.

MATERIALS AND METHODS

Intestinal permeability for ^{51}Cr-EDTA (Amersham International, Amersham, UK) was measured in 23 patients (14 female and nine male) with a median age of 33 yr (range 19–72 yr) with refractory active Crohn's disease, 2 days before and 4 wk after the first infusion of infliximab (Remicade, Centocor, Malvern, PA) 5 mg/kg. The patient data are shown in Table 1. Patients continued their treatment before and after the administration of anti-TNF-α.

Thirty-one healthy volunteers (18 female and 13 male) with a median age of 23 yr (range 21–50) performed the same test.

Clinical Scores

The Crohn's Disease Activity Index (CDAI) (29) was measured in the week preceding the permeability test and C-reactive protein (CRP) in serum was assessed on the day of the test. CDAI and CRP are expressed as median (interquartile range [IQR]).

Permeability Test

After an overnight fast the subjects drank 160 ml of Nutridrink (390 mOsm/L) (Nutricia, Bornem, Belgium) with 50 μCi ^{51}Cr-EDTA and rinsed the glass with 90 ml of water (30). Eating or drinking was not allowed for the next 2 h. Urine was collected for 24 h fractionated from 0–6 h and 6–24 h; the former measures permeation mainly in the small bowel, whereas the 0–24 h value is representative of whole gut permeation. Alcohol and nonsteroidal anti-inflammatory drug intake was prohibited in the 24 h and 1 wk, respec-

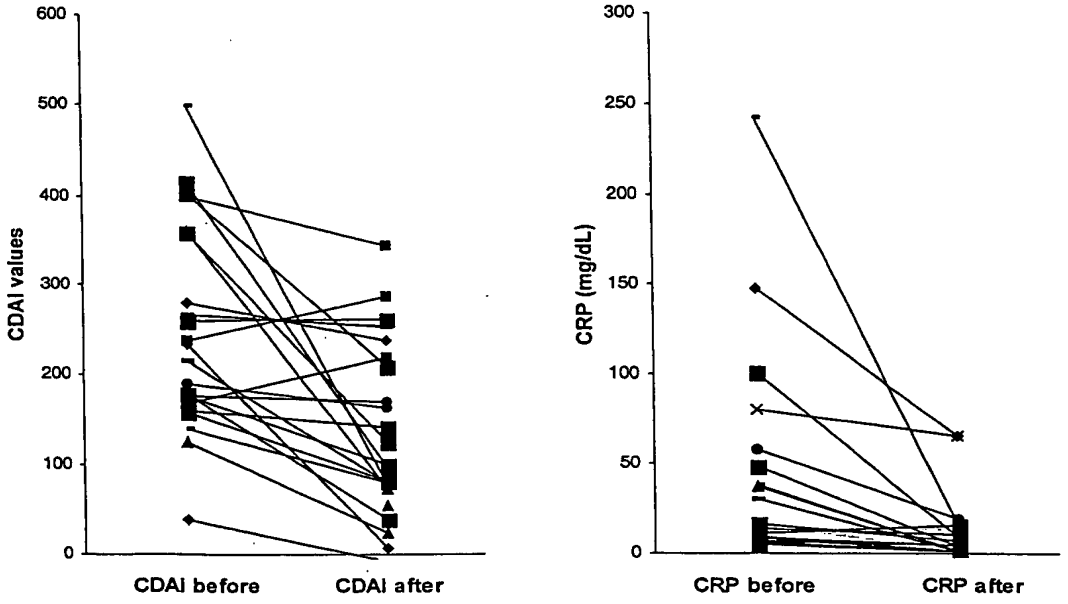

Figure 1. Individual values of CDAI and CRP before and after treatment.

Table 2. Median Permeability Values of Control Subjects and Crohn's Disease Patients

	% Dose Recovery, median (IQR)	
	0–6 h	0–24 h
Control values	1.12 (0.85–1.58)	2.28 (1.88–2.86)
Before anti-TNF	1.63 (1.06–2.07)*	3.27 (2.40–4.38)*
After anti-TNF	1.04 (0.74–1.54)†	2.42 (2.03–2.80)†

*Crohn's disease-patients compared to control subjects; $p = 0.05$.
† Before and after infliximab; $p = 0.05$.

tively, preceding and during the test. Volumes of urine were recorded and 1-ml aliquots were counted for radioactivity by a β liquid scintillation counter (Packard, model 2100, Downers Grove, IL) within 48 h after sampling. Results were expressed as the percent urinary excretion of the orally administered dose of ^{51}Cr-EDTA. Background radiation, defined as radiation measured in the absence of ^{51}Cr-EDTA, was negligible.

Statistical Analysis

Logarithmic transformation of the permeability values yielded normal distributions in the Crohn's disease and control groups. Results were considered statistically significant when the two-sided probability was less than 0.05. A paired *t* test was performed in the Crohn's disease group, along with an unpaired *t* test to compare the CD and control group permeability values. The CDAI values were not normally distributed and were compared with the Wilcoxon matched pairs test.

The CRP values had a normal distribution and were compared with a paired *t* test. Fisher exact test was used to evaluate the relation between permeability and treatment response. All tests were performed with the Statistica program on PC (StatSoft, Tulsa, OK).

Study Approval

The study was approved by the Ethical Committee of the University and all patients gave their written informed consent for the study.

RESULTS

The CDAI decreased significantly ($p < .01$) from 225 (170–264) to 99 (74–158) and CRP from 14.9 (9.1–43) to 3.7 (1–11.6) mg/L under anti-TNF treatment. The individual values are shown in Figure 1. Urinary volumes before and after infliximab treatment did not differ significantly. Intestinal permeation values for ^{51}Cr-EDTA were independent of the urinary volumes. Table 2 shows the results of intestinal permeabilities for ^{51}Cr-EDTA expressed as median percentage and interquartile range (p25–p75) for the patients before and after therapy and for normal controls. The small bowel and whole gut permeation of ^{51}Cr-EDTA (0–6 h and 0–24 h) in CD patients before treatment was significantly higher than in control subjects. The small intestinal and whole gut permeability in CD patients decreased significantly to normal mean values after treatment. The individual permeation values are shown in Figure 2. In the patients with very elevated permeation before therapy anti-TNF decreased permeation but did not completely bring it back to values comparable to those seen in normal volunteers. In contrast it is striking that, in most patients with permcation values that were within normal limits before therapy, anti-TNF still decreased permeation, suggesting that the absolute permeability values may not be so important.

The decrease in whole gut permeation for ^{51}Cr-EDTA paralleled clinical improvement and the improvement of acute phase reactants in 77% and 78% of the patients, respectively. Intestinal permeability did not correlate signif-

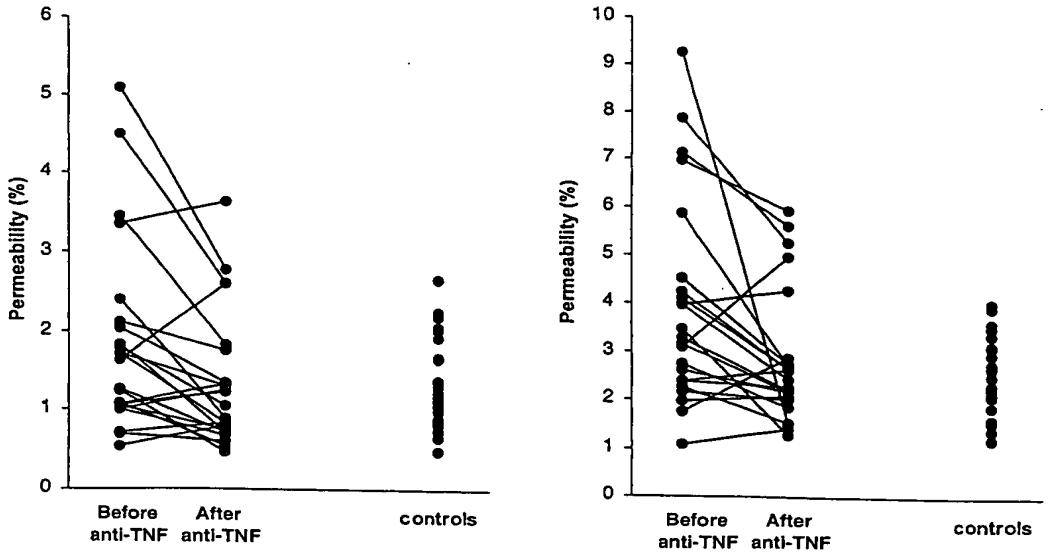

Figure 2. Individual small bowel (left) and whole gut permeability (right) values in CD patients and controls.

icantly with response outcome, probably because of the limited number of patients included.

DISCUSSION

The dramatic clinical effect of anti-TNF treatment in refractory Crohn's disease has clearly been shown by the pilot trial by Van Dullemen *et al.* (31) and the pivotal placebo controlled trial by Targan *et al.* (32). In patients responding to this anticytokine therapy, rapid and thorough endoscopic and histological healing of the bowel has been demonstrated (33). Moreover, immunohistochemical studies have shown important downregulation of proinflammatory cytokines and adhesion molecules in Crohn's disease mucosa (34).

The present study shows for the first time *in vivo* that neutralization of the proinflammatory cytokine TNF not only suppresses bowel inflammation but also largely restores the gut barrier in Crohn's disease, offering evidence for the hypothesis that the alterations of the gut barrier in CD are induced by proinflammatory cytokines.

It is clear from this study that suppression of inflammation and barrier restoration are both induced by the same cytokine antibody. This implies that inflammation and barrier function are closely interrelated. The hypothesis of a primary role of hyperresponsiveness of the gut barrier to gut flora or other exogenous factors in the initiation or relapse of the intestinal inflammation still may stand. Treatments such as an elemental diet (19–21) or prednisolone (22) have already shown to induce clinical remission and a decrease in gut permeability in active Crohn's disease. The precise importance of the present study lies in the fact that precise targeting by human chimeric monoclonal antibodies, shutting off one cytokine, is able to alter an essential property such as gut barrier function *in vivo*.

In the healthy normal bowel, "controlled inflammation" regulates contact with the potentially harmful luminal environment (35). In Crohn's disease there is an increased influx of inflammatory effector cells and upregulation of proinflammatory cytokines and other mediators of inflammation, and this delicate balance is disturbed. Cytokines such as interleukin-4 (36, 37), interferon-γ (IFN-γ) (38, 39) and TNF (40, 41) increase epithelial permeability through modulation of tight junction structure in intact monolayers. Serosal TNF decreased transepithelial resistance in the colonic epithelial cell line HT-29/B6 accompanied by a decrease in the number of tight junction strands, and adding IFN-γ to TNF further synergized in impairing the intestinal barrier (40). Zareie *et al.* demonstrated the capability of macrophages activated *in vitro* by *Salmonella minnesota* lipopolysaccharide and an *E. coli*-derived tripeptide, of mediating epithelial barrier function in T84 monolayers (41). The macrophage-derived TNF formed the key cytokine in these epithelial changes by means of an autocrine mechanism, as was shown by addition of a neutralizing antibody against TNF (41). TNF thus plays a critical role in modulating barrier function in IBD, through either direct or indirect action on the intestinal epithelium *in vitro*. The present *in vivo* study confirms that TNF exerts a significant effect on the intestinal barrier, and thus underlines the key role of TNF in the immunomodulation of gut permeability in inflammatory conditions.

In conclusion, we have shown that anti-TNF treatment largely restores gut barrier function in Crohn's disease. We hypothesise that the first event in the initiation of CD is aberrant activation of macrophages in the mucosa leading to increased production of proinflammatory cytokines responsible for disrupting the gut barrier. Permeation of gut flora components then further upregulates and perpetuates inflammation, leading to full-blown Crohn's disease.

Reprint requests and correspondence: P. Rutgeerts, M.D., Ph.D., F.R.C.P., Department of Gastroenterology, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium.

Received July 10, 2001; accepted Jan. 3, 2002.

REFERENCES

1. Shorter RG, Huizinga A, Spencer RT. A working hypothesis for the etiology and pathogenesis of IBD. Am J Dig Dis 1972;17:1024–32.
2. Madara JL, Pappenheimer JR. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. J Membr Biol 1987;100:149–64.
3. Marin ML, Greenstein AJ, Geller SA, et al. A freeze fracture study of Crohn's disease of the terminal ileum: Changes in epithelial tight junction organization. Am J Gastroenterol 1983;78:537–47.
4. Ramage JK, Stanisz A, Scicchitano R, et al. Effect of immunologic reactions on rat intestinal epithelium. Gastroenterology 1988;94:1368–75.
5. Bjarnason I, O'Morain C, Levi AJ, et al. Absorption of ^{51}chromium-labeled ethylenediaminetetraacetate in inflammatory bowel disease. Gastroenterology 1983;85:318–22.
6. Teahon K, Somasundaram S, Smith T, et al. Assessing the site of increased intestinal permeability in coeliac and inflammatory bowel disease. Gut 1996;38:864–69.
7. Jenkins RT, Jones DB, Goodacre RL, et al. Reversibility of increased intestinal permeability to ^{51}Cr-EDTA in patients with gastrointestinal inflammatory diseases. Am J Gastroenterol 1987;82:1159–64.
8. Jenkins RT, Ramage JK, Jones DB, et al. Small bowel and colonic permeability to ^{51}Cr-EDTA in patients with active inflammatory bowel disease. Clin Invest Med 1988;11:151–5.
9. Ainsworth M, Eriksen J, Rasmussen JW, et al. Intestinal permeability of ^{51}Cr-labelled ethylenediaminetetraacetic acid in patients with Crohn's disease and their healthy relatives. Scand J Gastroenterol 1989;24:993–8.
10. Pironi L, Miglioli M, Ruggeri E, et al. Relationship between intestinal permeability to [51Cr]EDTA and inflammatory activity in asymptomatic patients with Crohn's disease. Dig Dis Sci 1990;35:582–8.
11. Adenis A, Colombel JF, Lecouffe P, et al. Increased pulmonary and intestinal permeability in Crohn's disease. Gut 1992; 33:678–82.
12. Söderholm JD, Olaison G, Lindberg E, et al. Different intestinal permeability patterns in relatives and spouses of patients with Crohn's disease: An inherited defect in mucosal defence? Gut 1999;44:96–100.

13. Söderholm J, Peterson K, Olaison G, et al. Epithelial permeability to proteins in the noninflamed ileum of Crohn's disease? Gastroenterology 1999;117:65–72.
14. Peeters M, Ghoos Y, Maes B, et al. Increased permeability of macroscopically normal small bowel in Crohn's disease. Dig Dis Sci 1994;39:2170–6.
15. Hollander D, Vadheim C, Brettholz E, et al. Increased intestinal permeability in patients with Crohn's disease and their relatives. Ann Intern Med 1986;105:883–5.
16. Peeters M, Geypens B, Claus D, et al. Clustering of increased small intestinal permeability in families with Crohn's disease. Gastroenterology 1997;113:802–7.
17. May GR, Sutherland LR, Meddings JB. Is small permeability really increased in relatives of patients with Crohn's disease? Gastroenterology 1993;104:1627–32.
18. Hollander D. Permeability in Crohn's disease: Altered barrier functions in healthy relatives? Gastroenterology 1993;104: 1848–51.
19. Teahon K, Smethurst P, Levi AJ, et al. The effect of elemental diet on intestinal permeability and inflammation in Crohn's disease. Gastroenterology 1991;101:84–9.
20. Zoli G, Carè M, Parazza M, et al. A randomized controlled study comparing elemental diet and steroid treatment in Crohn's disease. Aliment Pharmacol Ther 1997;11:735–40.
21. Sanderson I, Boulton P, Menzies I, et al. Improvement of abnormal lactulose/rhamnose permeability in active Crohn's disease of the small bowel by an elemental diet. Gut 1987;28: 1073–6.
22. Miki K, Moore D, Butler R, et al. The sugar permeability test reflects disease activity in children and adolescents with inflammatory bowel disease. J Pediatr 1998;133:750–4.
23. Wyatt J, Vogels H, Hübl W, et al. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet 1993; 341:1437–9.
24. Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 1998;66:5224–31.
25. Rutgeerts P, Geboes K, Peeters M, et al. Effect of fecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 1991;338:771–4.
26. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998;114: 262–7.
27. Harper P, Lee E, Kettlewell M, et al. Role of faecal stream in the maintenance of Crohn's colitis. Gut 1985;26:279–84.
28. Fasoli R, Kettlewell M, Mortensen N, et al. Response to fecal challenge in defunctioned colonic Crohn's disease: Prediction of long-term response. Br J Surg 1990;77:616–7.
29. Best WR, Becktel JM, Singleton JW, et al. Development of a Crohn's disease activity index. Gastroenterology 1976;70: 439–44.
30. Peeters M, Hiele M, Ghoos Y, et al. Test conditions greatly influence permeation of water soluble molecules through the intestinal mucosa: Need for standardisation. Gut 1994;35: 1404–18.
31. van Dullemen HM, van Deventer S, Hommes D, et al. Treatment of Crohn's disease with anti-TNF chimeric monoclonal antibody (cA2). Gastroenterology 1995;109:129–35.
32. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med 1997;337:1029–35.
33. D'Haens G, Van Deventer S, Van Hogezand R, et al. Endoscopic and histological healing with infliximab anti-TNF antibodies in Crohn's disease: A European multicenter trial. Gastroenterology 1999;116:1029–34.
34. Baert F, D'Haens G, Peeters M, et al. Tumor necrosis factor α (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. Gastroenterology 1999;116:22–8.
35. Mowat AM, Viney JL. The anatomical basis of intestinal immunity. Immunol Rev 1997;156:145–66.
36. Colgan SP, Resnick MB, Parkos CA, et al. IL-4 directly modulates function of a model human intestinal epithelium. J Immunol 1994;153:2122–9.
37. Zund G, Madara JL, Dzus AL, et al. Interleukin-4 and interleukin-13 differentially regulate epithelial chloride secretion. J Biol Chem 1996;271:7460–4.
38. Youakim A, Minoo A. Interferon-γ decreases barrier function in T84 cells by reducing ZO-1 levels and disrupting apical actin. Am J Physiol 1999;276:G1279–88.
39. McKay DM, Singh PK. Superantigen activation of immune cells evokes epithelial (T84) transport and barrier abnormalities via IFN-γ and TNF: Inhibition of increased permeability, but not diminished secretory responses by TGF-β2. J Immunol 1997;159:2382–90.
40. Schmitz H, Fromm M, Bentzel CJ, et al. Tumor necrosis factor-alpha (TNF-α) regulates the epithelial barier in the human intestinal cell line HT-29/B6. J Cell Sci 1999;112:137–46.
41. Zareie M, McKay D, Kovarik G, et al. Monocyte/macrophage evoke epithelial dysfunction: Indirect role of tumor necrosis factor-α. Am J Physiol 1998;275:C932–9.